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Original Article

Comparative toxicity study on classical and modified version of Jawarish Jalinoos (a traditional Unani formulation) in rats

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ABSTRACT

Background: Jawarish Jalinoos (JJ) is a classical semisolid traditional Unani formulation clinically used for the treatment of weakness of vital organs, liver, and stomach. Although JJ has been widely used clinically for several decades, no scientific report is available for its safety.
Methods: JJ and its sugar-free tablet version (SFJJ; formulated to target diabetic population) were assessed for safety in rats. Ninety-day repeated dose oral toxicity study was performed as per the Organisation for Economic Co-operation and Development Guideline 408. JJ was orally administered at the dose of 2000 mg/kg bw/d, whereas SFJJ was orally administered at the doses of 506 mg/kg body weight (bw)/d, 1012 mg/kg bw/d, and 2024 mg/kg bw/d for 90 days. The animals were periodically observed for clinical signs of toxicity, mortality, morbidity, body weight changes, and feed consumption. At the end of the study, hematology, clinical biochemistry, electrolytes, gross pathology, relative organ weight, and histological examination were performed.
Results: Treatment with SFJJ and JJ showed no significant differences in body weight gain, feed consumption, hematology, clinical biochemistry, and serum electrolytes. No gross pathological findings and differences in relative organ weights were observed between control and drug treated rats. Histological examination revealed no toxicologically significant abnormalities related with SFJJ or JJ treatment.
Conclusion: The 90-day repeated dose oral toxicity study demonstrates that the no observed adverse effect level of SFJJ and JJ is greater than 2024 mg/kg bw/d and 2000 mg/kg bw/d (p.o.) in rats, respectively. Both formulations were found to be safe up to the tested dose levels and experimental conditions, and therefore safe for clinical use as specified in the literature.

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1. Introduction

The Unani system of medicine is one of the most ancient systems of medicine and has established its therapeutic importance for a long time.[1,2] The Unani system of medicine is a comprehensive medical system that meticulously deals with the various states of health and disease.[1,2] There are many diseases for which the Unani system has highly effective treatments; however, they require optimization and testing as per current regulatory scenario in order to scientifically validate these valuable medicines. Furthermore, there is a need to develop convenient dosage forms with ease of administration and optimization of dose uniformity. With the change in era, Western medicine met with continuous advancements in terms of pharmaceutical drug delivery systems, formulation techniques, and regulatory requirements, whereas the Unani system has still to achieve the same pace of progress. The efficacy of traditional Unani formulations has been well proven through clinical evaluation as well as their widespread use throughout the centuries. However, there is a need to establish the safety of such Unani formulations to surpass the scrutiny of regulatory framework as well as to offer these valuable medicines to a larger population across the globe.

Jawarish Jalinoos (JJ) is a classical semisolid Unani formulation having Muqawwi-e-Aam (general tonic), Kasir-e-Riyah (carminative), Muqawwi-e-Bah (aphrodisiac), and Hazim (digestive) actions. JJ is indicated for the management of Zof-e-Aza-e-Raeesa (weakness of vital organs), Zof-e-Meda (weakness of stomach), Zof-e-Kabid (weakness of liver), Khafqan (palpitation), Dard-e-Sar (headache), Khansi (cough), Bawaseer (hemorrhoids), Niqris (gout), Daad (eczema), Kasrat-e-Baul (polyurea), and Hissat-e-Kulliya wo Masana (urinary stones).[3,4] JJ is also effective in headaches, excessive micturition, and maintains the blackness of hair.[5] The classical version of JJ has been used clinically for decades without any significant adverse effect reported so far. However, the classical semisolid preparation of JJ contains a high quantity of honey/sugar, which limits the widespread clinical use of this highly efficacious formulation in the diabetic population. Therefore, redesigning of this classical formulation into a sugar-free version was planned to widen its scope for diabetic patients as well as for dose reduction. Furthermore, the sugar-free tablet version (SFJJ) is expected to improve patient compliance as a tablet version is easy to administer and more convenient compared to the classical semisolid preparation. SFJJ has to undergo preclinical safety assessment prior to entering into clinical therapeutic evaluation. Therefore, the preclinical toxicity of a sugar-free tablet version as well as classical semisolid version is evaluated in Sprague–Dawley (SD) rats in the present study.

2. Methods

2.1. Experimental animals

SD rats (100 ± 20 g) were obtained from the National Institute of Nutrition, Hyderabad, India. The selected females were nulliparous and nonpregnant. The rats were housed in polycarbonate cages in an air-conditioned room maintained at a temperature of $22 \pm 3\,^{\circ}$C and relative humidity of 30–70%, with a 12:12 hour light/dark illumination cycle.[6] CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines of laboratory animal care were followed throughout the experiment.[7] The protocol used in this study was approved by the Institutional Animals Ethics Committee vide protocol no. CRIUM/IAEC/2014/02/P01. The animals were provided with standard feed pellets (National Institute of Nutrition, Hyderabad, India) and water *ad libitum*, unless stated otherwise. Then they were acclimatized to the laboratory conditions for 1 week prior to the experiment.

2.2. Drug/formulation

The classical semisolid version of JJ used in the present study was prepared as per the standard Pharmacopoeial procedure in the Good Manufacturing Practice-certified pharmacy of the Central Research Institute of Unani Medicine (CRIUM) Hyderabad.[3] SFJJ was prepared at the National Institute of Pharmaceutical Education and Research (NIPER) Hyderabad by removing the sugar from the classical Pharmacopoeial formulae and incorporating permitted additives so that tablets can be made using the compression technique.

2.3. Dose selection

The recommended therapeutic dose of JJ in Unani system of medicine is 5–15 g/d. Therefore, as a conservative approach, 15 g/d was considered as adult dose, and we eventually arrived at a dose of 1542 mg/kg of classical JJ for rats after translating the human adult dose based on body surface area.[8] As per ICH guidelines, a limited dose of 2000 mg/kg body weight (bw)/d was used for the classical version of JJ as the therapeutic equivalent dose is already 1542 mg/kg bw, and other groups with higher doses were not feasible.[9] For the sugar-free tablet version, 4.92 g of SFJJ is equivalent to 15 g of the classical version after the removal of sugar. Therefore, a dose of 506 mg/kg was considered appropriate for rats after translating the therapeutic equivalent dose of SFJJ (i.e., 4.92 g) based on body surface area. Accordingly, SFJJ was administered at three dose levels: 506 mg/kg bw/d, 1012 mg/kg bw/d, and 2024 mg/kg bw/d (i.e., 1×, 2×, and 4× of therapeutic equivalent dose, respectively).

2.4. Vehicle

Aqueous carboxymethyl cellulose (CMC; 0.3%) suspension was used as vehicle for the oral administration of both formulations.

2.5. Drug administration

Both formulations were suspended in 0.3% aqueous CMC using mortar pestle. Drugs or vehicle were orally administered via a stainless steel gavage, by calculating the dose based on the body weight of rats, for a period of 90 days. Duration of toxicity study for JJ was decided as 3 months (i.e., 90 days) based on its duration of clinical use in the Unani system.

2.6. *Experimental design*

The 90-day repeated dose oral toxicity study was performed according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline 408.[6] Male and female SD rats were divided into five groups of 20 animals (10 males + 10 females) each as follows:

- Vehicle control (0.3% aqueous CMC)
- SFJJ 506 mg/kg bw/d (1×; therapeutically equivalent dose)
- SFJJ 1012 mg/kg bw/d (2×)
- SFJJ 2024 mg/kg bw/d (4×)
- JJ 2000 mg/kg bw/d

All experimental animals were observed for mortality and morbidity twice a day, throughout the study duration. Detailed clinical observations (i.e., functional observation parameters) were made periodically to detect signs of toxicity, at the same time (1 hour after vehicle or drug administration). Body weight of the animals was recorded once a week. Average feed intake for both sexes was recorded at weekly interval by weighing the amounts of feed given to a cage group and leftovers on the next day. At the end of the treatment period, the overnight-fasted (water provided *ad libitum*) rats were anesthetized with isofluorane inhalation (EZ Anaesthesia-1339). Then, blood samples were collected by retro-orbital puncture in the EDTA vacutainers (for hematological) and serum vacutainers (for biochemical and electrolyte analysis).

Hemoglobin (Hb), red blood cell count (RBC), white blood cell count (WBC), hematocrit (HCT), and platelet (PLT) were analyzed using a fully automated hematology analyzer (Swelab Autocounter-920EO+). Serum biochemical parameters such as glucose, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total bilirubin, creatinine, blood urea nitrogen (BUN), total cholesterol (TC), triglycerides, total protein (TP), and albumin were analyzed using a fully automatic analyzer (Erba-EM200). Serum electrolytes such as sodium, potassium, chloride, and total calcium were also estimated using a fully automated electrolyte analyzer (Allcare-AC9801).

In the last week (13th week) of the drug treatment, all rats were subjected to rotarod test to evaluate locomotor coordination. Briefly, a habituation session was performed initially, and rats were placed on a rod rotating at a speed of 12 revolutions/min for a period of 2 minutes (Ugobasile-47700, Italy). If they fall off during this period, they were placed again on the rod. On the next day of the habituation trial, rats were placed on the rod under the same conditions as used during the habituation session and were not placed back on the rotarod if they fall off. The latency to fall off from rotating rod was recorded (maximum, 3 minutes).[10] Furthermore, animals were also subjected to grip strength test using a computerized grip strength meter (GSM; Orchid Scientifics DSM-I, Nashik, India). Briefly, a rat held by the tail was placed on a small metallic grid, which the animal gripped with its forepaws and pulled backward. As soon as the animal released the grip, the maximum force (i.e., peak force) was recorded by the control unit.

At the end of the study, all animals were subjected to gross necropsy. Organs and tissues were examined macroscopi-

Table 1 – Effect of JJ and SFJJ on hematological parameters in rats.

Sex		Female (n = 10)					Male (n = 10)					Female + male (n = 20)				
Daily dose (mg/kg bw/d)		Control (CMC)	SFJJ (506)	SFJJ (1012)	SFJJ (2024)	JJ (2000)	Control (CMC)	SFJJ (506)	SFJJ (1012)	SFJJ (2024)	JJ (2000)	Control (CMC)	SFJJ (506)	SFJJ (1012)	SFJJ (2024)	JJ (2000)
Hemoglobin	gm%	16.34 ± 0.313	17.33 ± 0.164	16.68 ± 0.60	16.94 ± 0.394	16.98 ± 0.153	17.37 ± 0.276	17.41 ± 0.441	18.12 ± 0.711	17.68 ± 0.214	18.19 ± 0.459	16.855 ± 0.235	17.37 ± 0.229	17.40 ± 0.354	17.18 ± 0.220	17.59 ± 0.273
RBC	million/mm^3	8.14 ± 0.122	7.77 ± 0.307	8.02 ± 0.186	8.038 ± 0.465	8.64 ± 0.043	8.93 ± 0.091	8.110 ± 0.172*	8.26 ± 0.548	9.025 ± 0.165	8.48 ± 0.174	8.535 ± 0.117	7.94 ± 0.176	8.14 ± 0.195	8.367 ± 0.262	8.56 ± 0.089
WBC	/mm^3	6460 ± 542.3	7290 ± 483.80	5280 ± 267.28	5850 ± 457.50	7570 ± 338.30	6170 ± 362.7	7100 ± 335	7525 ± 915.94	4800 ± 308.20	7490 ± 553.50	6315 ± 319.23	7195 ± 287.23	6278 ± 376.62	5500 ± 267.98	7530 ± 315.82
Platelets	Lakhs/mm^3	4.28 ± 0.103	5.250 ± 0.335*	4.84 ± 0.271	3.40 ± 0.263	4.78 ± 0.173	3.89 ± 0.148	4.79 ± 0.269	5.360 ± 0.638*	3.575 ± 0.409	4.80 ± 0.141	4.085 ± 0.098	5.02 ± 0.216**	5.10 ± 0.239*	3.458 ± 0.165	4.79 ± 0.109
HCT	%	44.24 ± 0.414	45.78 ± 1.808	44.36 ± 0.761	44.00 ± 2.628	47.80 ± 0.448	46.51 ± 0.588	45.33 ± 0.818	45.40 ± 2.941	47.58 ± 0.809	45.37 ± 0.943	45.38 ± 0.436	45.56 ± 0.967	44.88 ± 1.020	45.19 ± 1.396	46.59 ± 0.580

Values presented as mean ± SEM; n = 20 (10/sex); one-way ANOVA.
ANOVA, analysis of variance; bw, body weight; CMC, carboxymethyl cellulose; HCT, hematocrit; JJ, Jawarish Jalinoos; RBC, red blood cell count; SEM, standard error of the mean; SFJJ, sugar-free tablet version of JJ; WBC, white blood cell count.
*$p < 0.05$; **$p < 0.01$ compared to control.

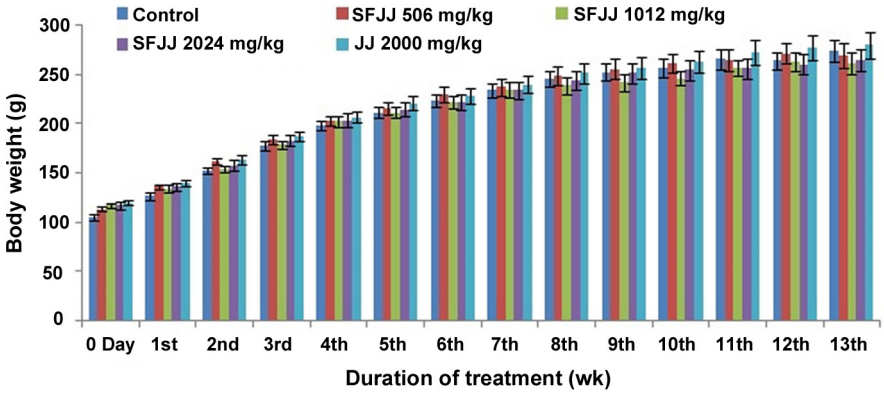

Fig. 1 – Effect of SFJJ and JJ on Body Weight in Rats (female + male); one-way ANOVA. No statistically significant differences were found compared to control ($n = 20$).
Data are presented as mean ± SEM.
ANOVA, analysis of variance; JJ, Jawarish Jalinoos; SEM, standard error of the mean; SFJJ, sugar-free tablet version of JJ.

cally, and internal organs/tissues were isolated, trimmed, and weighed. Organs/tissues were preserved in neutral buffered formalin and subjected to histological examination.

2.7. *Statistical analyses*

Data were expressed as mean ± standard error of mean. The mean difference between the control and treatment groups was analyzed by one-way analysis of variance followed by Tukey's multiple comparison as *post hoc* test using GraphPad prism (version 5) GraphPad Software, Inc., CA, USA. A p value ≤ 0.05 was considered statistically significant.

3. Results

Clinical examinations of rats made at different time intervals did not reveal any incidence of abnormal clinical signs/behavior suggestive of any systemic toxicity among the rats treated with SFJJ at the dose levels of 506 mg/kg, 1012 mg/kg, and 2024 mg/kg or 2000 mg/kg of the classical JJ version or control group animals. All the rats in the study survived throughout the 90-day study period. No significant difference in body weight gain was observed between the control and drug-treated groups during the study (Fig. 1). There was no statistically significant difference noted in feed intake of any drug-treated groups in any sex compared to the vehicle-treated control rats (Fig. 2). Hematological parameters such as Hb, RBC, WBC count, and HCT of drug-treated rats were comparable to those of control rats. There were significant increase ($p < 0.05$) in PLT count only in the low dose SFJJ females and mid dose SFJJ males; however, values remained within the normal physiological range of SD rats (Table 1). No dose-dependent treatment-related significant difference was observed in blood biochemical parameters such as AST, ALT, ALP, TP, albumin, bilirubin, creatinine, and BUN between control and drug-treated groups (Table 2). Blood glucose level was significantly lower ($p < 0.05$) in mid dose females of SFJJ but not

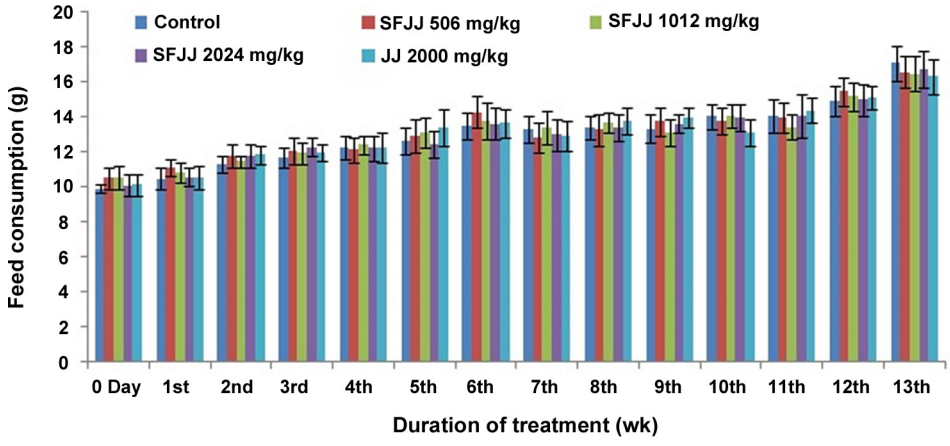

Fig. 2 – Effect of SFJJ and JJ on feed consumption in rats (female + male); one-way ANOVA. No statistically significant differences were found compared to control ($n = 20$).
Data are presented as mean ± SEM.
ANOVA, analysis of variance; JJ, Jawarish Jalinoos; SEM, standard error of the mean; SFJJ, sugar-free tablet version of JJ.

Table 2 – Effect of SFJJ and JJ on blood biochemical parameters in rats.

Sex		Female ($n = 10$)					Male ($n = 10$)					Female + male ($n = 20$)				
Daily dose (mg/kg bw/d)		Control (CMC)	SFJJ (506)	SFJJ (1012)	SFJJ (2024)	JJ (2000)	Control (CMC)	SFJJ (506)	SFJJ (1012)	SFJJ (2024)	JJ (2000)	Control (CMC)	SFJJ (506)	SFJJ (1012)	SFJJ (2024)	JJ (2000)
AST	IU/L	135.40 ± 13.73	142.50 ± 6.64	135.60 ± 19.00	163.10 ± 10.30	119.60 ± 10.33	143.70 ± 15.66	155.80 ± 7.58	148.90 ± 22.79	155.80 ± 12.95	131.90 ± 8.79	139.55 ± 10.18	149.15 ± 5.14	142.25 ± 14.52	159.45 ± 8.10	125.75 ± 6.75
ALT	IU/L	70.40 ± 3.97	68.20 ± 4.48	68.60 ± 4.32	77.30 ± 8.32	68.30 ± 6.00	68.40 ± 6.43	88.30 ± 5.54	72.80 ± 3.87	83.40 ± 6.25	66.40 ± 3.95	69.40 ± 3.68	78.25 ± 4.17	70.70 ± 2.86	80.35 ± 5.11	67.35 ± 3.50
Bilirubin Total	mg/dL	0.237 ± 0.037	0.309 ± 0.017	0.244 ± 0.031	0.166 ± 0.028	0.291 ± 0.033	0.240 ± 0.056	0.270 ± 0.016	0.168 ± 0.012	0.217 ± 0.022	0.283 ± 0.025	0.239 ± 0.032	0.290 ± 0.012	0.206 ± 0.018	0.192 ± 0.018	0.287 ± 0.020
Alkaline phosphatase	IU/L	88.60 ± 10.89	117.10 ± 19.54	100.80 ± 5.29	113.70 ± 14.08	78.30 ± 4.89	100.60 ± 13.29	136.20 ± 7.77	106.20 ± 10.59	136.70 ± 15.98	83.10 ± 8.06	94.60 ± 8.47	126.65 ± 10.47	103.50 ± 5.79	125.20 ± 10.70	80.70 ± 4.62
Total protein	g/dL	6.44 ± 0.173	5.91 ± 0.20	5.83 ± 0.177	5.96 ± 0.174	7.05 ± 0.119	6.510 ± 0.156	5.81 ± 0.278	6.20 ± 0.207	6.10 ± 0.082	6.28 ± 0.088	6.48 ± 0.114	5.860 ± 0.165**	6.015 ± 0.139	6.030 ± 0.095	6.665 ± 0.114
Albumin	g/dL	3.510 ± 0.091	3.540 ± 0.052	3.660 ± 0.056	3.570 ± 0.060	3.740 ± 0.064	3.398 ± 0.096	3.610 ± 0.060	3.660 ± 0.082	3.398 ± 0.119	3.430 ± 0.084	3.454 ± 0.066	3.575 ± 0.040	3.660 ± 0.048	3.484 ± 0.068	3.585 ± 0.063
Blood urea nitrogen	mg/dL	18.26 ± 1.125	15.14 ± 1.135	16.67 ± 0.334	17.69 ± 1.127	18.89 ± 0.662	16.02 ± 1.165	12.30 ± 0.991	14.44 ± 1.183	11.78 ± 1.450	14.94 ± 0.689	17.14 ± 0.829	13.72 ± 0.802	15.56 ± 0.651	14.74 ± 1.122	16.92 ± 0.649
Creatinine	mg/dL	0.822 ± 0.022	0.780 ± 0.013	0.849 ± 0.009	0.810 ± 0.010	0.800 ± 0.026	0.780 ± 0.011	0.754 ± 0.014	0.827 ± 0.016	0.758 ± 0.029	0.788 ± 0.028	0.801 ± 0.013	0.767 ± 0.010	0.838 ± 0.009	0.784 ± 0.016	0.794 ± 0.018
Glucose	mg/dL	110.20 ± 6.11	116.30 ± 3.58	85.70 ± 5.56*	107.30 ± 4.43	120.90 ± 7.46	103.60 ± 5.64	121.90 ± 8.096	89.60 ± 5.47	114.30 ± 6.40	102.40 ± 7.23	106.90 ± 4.12	119.10 ± 4.36	87.65 ± 3.82*	110.80 ± 3.87	111.650 ± 5.48
Cholesterol	mg/dL	90.50 ± 7.27	129.50 ± 1.93***	107.10 ± 3.73	95.60 ± 5.10	114.90 ± 4.04**	82.40 ± 6.57	96.50 ± 1.29	77.10 ± 2.64	75.10 ± 3.47	103.4 ± 2.26**	86.45 ± 4.86	113.00 ± 3.95***	92.10 ± 4.10	85.35 ± 3.81	109.15 ± 2.61**
Triglycerides	mg/dL	60.70 ± 4.24	78.40 ± 12.45	64.70 ± 4.47	66.50 ± 2.74	65.10 ± 4.52	62.90 ± 6.85	79.80 ± 7.67	78.40 ± 3.32	80.60 ± 2.79	56.80 ± 3.43	61.80 ± 3.93	79.10 ± 7.12	71.55 ± 3.13	73.55 ± 2.50	60.95 ± 2.92

Values presented as mean ± SEM; $n = 20$ (10/sex); one-way ANOVA.

ALT, alanine transaminase; ANOVA, analysis of variance; AST, aspartate transaminase; bw, body weight; CMC, carboxymethyl cellulose; JJ, Jawarish Jalinoos; SEM, standard error of the mean; SFJJ, sugar-free tablet version of JJ.

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ compared to control.

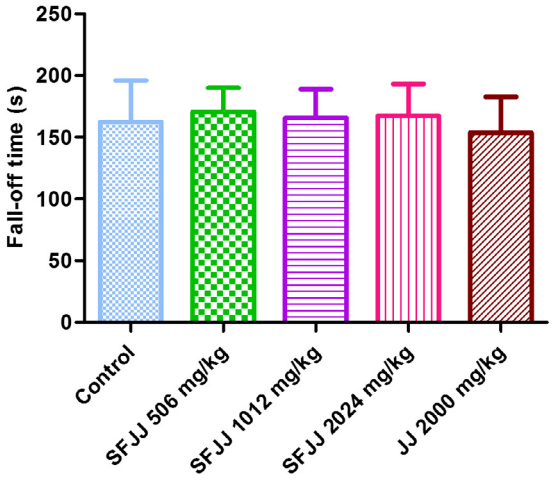

Fig. 3 – Effect of SFJJ and JJ on the rotarod performance in rats (female + male); one-way ANOVA. No statistically significant differences were found compared to control ($n = 20$).
Data are presented as mean ± SEM.
ANOVA, analysis of variance; JJ, Jawarish Jalinoos; SEM, standard error of the mean; SFJJ, sugar-free tablet version of JJ.

in the other dose groups, and hence may not be considered toxicologically significant. Similarly, the TC was significantly higher only in low dose of SFJJ group females ($p < 0.001$) and JJ classical group (both sexes; $p < 0.01$). Serum electrolytes such as sodium, potassium, chloride, and total calcium were found to be within the normal limits in the experimental and control groups, except for a few changes that were not dose-dependent, and hence may not be considered toxicologically significant (Table 3).

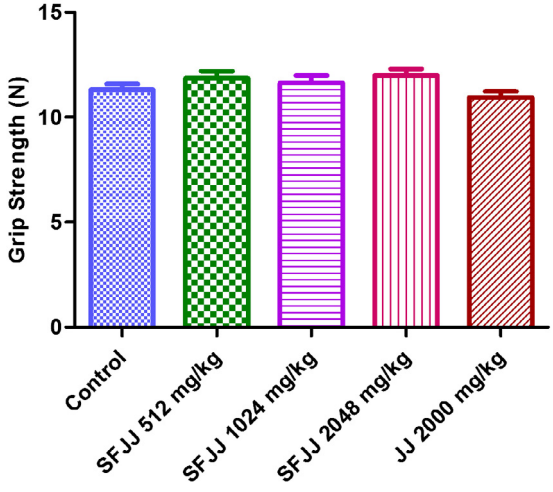

Fig. 4 – Effect of SFJJ and JJ on grip strength in rats (female + male); one-way ANOVA. No statistically significant differences were found compared to control ($n = 20$).
Data presented as mean ± SEM.
ANOVA, analysis of variance; JJ, Jawarish Jalinoos; SEM, standard error of the mean; SFJJ, sugar-free tablet version of JJ.

Table 3 – Effect of SFJJ and JJ on serum electrolytes in rats.

Sex		Female ($n = 10$)					Male ($n = 10$)					Female + male ($n = 20$)				
Daily dose (mg/kg bw/d)		Control (CMC)	SFJJ (506)	SFJJ (1012)	SFJJ (2024)	JJ (2000)	Control (CMC)	SFJJ (506)	SFJJ (1012)	SFJJ (2024)	JJ (2000)	Control (CMC)	SFJJ (506)	SFJJ (1012)	SFJJ (2024)	JJ (2000)
Sodium	mmol/L	139.60 ± 0.72	145.00 ± 3.56	144.10 ± 1.99	134.90 ± 2.47	137.70 ± 1.51	139.30 ± 1.10	147.50 ± 0.95	146.30 ± 1.99	140.80 ± 4.27	139.20 ± 0.60	139.45 ± 0.64	146.25 ± 1.82*	145.20 ± 1.39	137.85 ± 2.49	138.45 ± 0.81
Potassium	mmol/L	4.39 ± 0.176	4.88 ± 0.150	4.18 ± 0.141	4.41 ± 0.142	4.14 ± 0.083	4.91 ± 0.222	4.55 ± 0.043	4.63 ± 0.115	4.33 ± 0.115*	5.01 ± 0.108	4.65 ± 0.150	4.72 ± 0.085	4.41 ± 0.102	4.37 ± 0.089	4.58 ± 0.120
Chloride	mmol/L	105.60 ± 0.72	112.90 ± 1.55*	111.90 ± 1.27	100.60 ± 2.79	109.60 ± 1.01	106.80 ± 1.02	110.30 ± 1.01	109.10 ± 1.52	104.90 ± 3.45	106.60 ± 0.64	106.20 ± 0.62	111.60 ± 0.95*	110.50 ± 1.01	102.75 ± 2.22	108.10 ± 0.68
Calcium	mmol/L	2.87 ± 0.151	2.02 ± 0.023	2.93 ± 1.049	4.04 ± 0.106	2.57 ± 0.030	2.45 ± 0.182	2.14 ± 0.093	5.19 ± 1.350	3.54 ± 1.322	2.33 ± 0.040	2.66 ± 0.125	2.08 ± 0.048	4.06 ± 0.871	3.79 ± 0.648	2.45 ± 0.037

Values presented as mean ± SEM; $n = 20$ (10/sex); one-way ANOVA.
*$p < 0.05$ compared to control.
ANOVA, analysis of variance; bw, body weight; CMC, carboxymethyl cellulose; JJ, Jawarish Jalinoos; SEM, standard error of the mean; SFJJ, sugar-free tablet version of JJ.

There were no significant differences observed in motor performance in rotarod and grip strength (as assessed by GSM) between drug-treated and control animals (Figs. 3 and 4, respectively).

Furthermore, no significant differences were observed in the relative organ weight of brain, heart, thymus, liver, lungs, spleen, kidneys, adrenals, testes/ovaries, and uterus between the control and drug-treated groups (Table 4). No gross pathological changes were observed during necropsy in any group. Changes in histological significance (such as focal lobular inflammation) were observed only in the liver of one rat in the SFJJ group and two rats in the JJ classical group. Although foreign body granulomas were observed in the lungs of three animals in the JJ classical group, the changes could not be considered significant as they may have been caused by inhalation of some foreign matter by the animals. The histological changes observed in the rest of the organs of the experimental group were also observed in the vehicle control group and hence were not considered significant. Representative photomicrographs of the histological section of liver, kidney, heart, lung, spleen, pancreas, thymus, thyroid, parathyroid, trachea, esophagus, sternum with bone marrow, adrenals, skeletal muscle, small intestine, rectum, ovary, and testis of the control and high dose treated SFJJ and JJ groups are presented in Figs. 5A–5C.

4. Discussion

JJ is a polyherbal compound formulation widely used in the Unani system of medicine for the treatment of weakness of vital organs, liver, and stomach. Herbal medicines, like any other medicines, are not free of risk, and many studies suggest potential adverse reactions and interactions with herbal drugs. Available statistics show that some herbal products, used in traditional medication for generations, may possess hepatotoxic, cardiotoxic, and carcinogenic effects, as well as other severe actions.[11] Therefore, it is worthwhile to establish the safety of commonly used Unani formulations with widespread therapeutic applications. Several approaches have been adopted to assess the safety of novel drugs. Animal models may be helpful in understanding the safety risks of compounds with emphasis to understand the mechanism of a particular toxicity.[12]

The present study provides detailed information on the safety profile of SFJJ and classical JJ following repeated oral administration for 90 days in SD rats. No mortality was observed in any control or drug-treated group of animals throughout the study duration of 90 days, and none of the animals showed any signs of major or significant intoxication during the study period. Drug-treated rats of both sexes exhibited the expected pattern and comparable body weight gain and feed consumption with that of controls throughout the dosing period, suggesting normal growth and development. Body weight gain and feed consumption are nonspecific, broad screen for adverse systemic toxicity.[13] Functional observation test conducted at different time points did not reveal any drug treatment-related abnormalities.

Hematological and biochemical analyses conducted at the termination of study revealed no dose-dependent treatment-related alterations, and all values remained well within the normal physiological range.[7] The hemopoietic system is an important target for several xenobiotics and is a sensitive indicator for pathological conditions.[14] In the present study, treatment with SFJJ or JJ did not produce any alteration in hematological parameters (i.e., RBC, WBC, Hb, HCT), which indicated that the treatment did not affect the blood cells or their production. Significant increase in PLT count was only observed in low dose SFJJ females and mid dose SFJJ males. The PLT count remained well within the normal physiological range for rats,[7] and similar findings were not observed in the high dose group of SFJJ; hence, they could not be considered toxicologically relevant.

Hepatotoxicity is a leading cause of attrition in drug development or restricted use after marketing.[15] One of the most frequent reasons for the withdrawal from the market of an approved drug during the past decade is liver toxicity.[16] Liver plays a central role in the metabolism and excretion of xenobiotics, which makes it highly susceptible to the adverse and toxic effects of xenobiotics. Liver injury can be diagnosed by certain biomarkers such as ALT, AST, ALP, and bilirubin.[17] Elevations in serum enzyme levels are taken as relevant indicators of liver toxicity, whereas increases in both total and conjugated bilirubin levels are measures of overall liver function. Increases in the levels of ALT and AST in serum, in combination with increased bilirubin levels, are actually considered to be the most relevant indicator of liver toxicity.[16] Any elevation pertaining to ALT and AST indicate their outflow into the bloodstream owing to damage in liver parenchymal cells.[18,19] In the present investigation, the parameters of liver and renal functions were found to be well within the clinical range of rats in the drug- or vehicle-treated groups, which indicates that SFJJ or JJ did not affect liver function or metabolism.

Early detection of drug-induced kidney injury is critical in drug development.[20] The vital role of the kidney in drug excretion and detoxification makes it one of the major organs evoking drug-related toxic responses and an important target of toxicological studies. The standard parameters in preclinical or clinical trials for the detection and monitoring of renal function are serum creatinine and BUN.[21] In the present study, no treatment-related changes were observed in serum creatinine or BUN, suggesting that SFJJ and JJ are nontoxic to renal function. Although there is a significant increase in the TC level, observed in the group of low dose SFJJ females and the JJ classical group (both sexes), the values remained within normal physiological limits.[7] Fasting blood glucose level was significantly low compared to control rats only in mid dose SFJJ females, and no dose-dependent alteration in the blood glucose level was observed. Furthermore, except for a marginal increase in the chloride level of low dose SFJJ females (no effect on other dose groups of SFJJ), the serum electrolyte levels were found to be well within the normal physiological range of rats, which reflects that SFJJ and JJ have no adverse effect on electrolyte homeostasis.

Motor coordination has traditionally been assessed in rodents by the rotarod test, in which the animal is placed on a horizontal rod that rotates about its long axis; the animal must walk forward to remain upright and not fall off.[22] Muscular strength or neuromuscular function in rodents may

Table 4 – Effect of SFJJ and JJ on relative organ weight in rats.

Sex		Female ($n = 10$)					Male ($n = 10$)					Female + male ($n = 20$)				
Daily dose (mg/kg bw/d)		Control (CMC)	SFJJ (506)	SFJJ (1012)	SFJJ (2024)	JJ (2000)	Control (CMC)	SFJJ (506)	SFJJ (1012)	SFJJ (2024)	JJ (2000)	Control (CMC)	SFJJ (506)	SFJJ (1012)	SFJJ (2024)	JJ (2000)
Brain		0.814 ± 0.012	0.826 ± 0.020	0.838 ± 0.012	0.846 ± 0.020	0.808 ± 0.014	0.557 ± 0.015	0.602 ± 0.014	0.623 ± 0.016	0.613 ± 0.016	0.563 ± 0.008	0.686 ± 0.031	0.714 ± 0.028	0.730 ± 0.026	0.729 ± 0.030	0.686 ± 0.029
Thymus		0.120 ± 0.004	0.096 ± 0.004	0.103 ± 0.003	0.100 ± 0.005	0.103 ± 0.006	0.086 ± 0.006	0.079 ± 0.008	0.079 ± 0.010	0.069 ± 0.003	0.071 ± 0.005	0.103 ± 0.005	0.087 ± 0.005	0.091 ± 0.006	0.085 ± 0.005	0.087 ± 0.005
Heart		0.342 ± 0.007	0.340 ± 0.004	0.333 ± 0.009	0.341 ± 0.007	0.368 ± 0.010	0.312 ± 0.009	0.320 ± 0.008	0.312 ± 0.008	0.311 ± 0.003	0.361 ± 0.029	0.327 ± 0.006	0.330 ± 0.005	0.322 ± 0.006	0.326 ± 0.005	0.365 ± 0.015
Lungs		0.705 ± 0.053	0.652 ± 0.028	0.549 ± 0.034	0.569 ± 0.011	0.690 ± 0.052	0.717 ± 0.136	0.553 ± 0.039	0.598 ± 0.075	0.530 ± 0.035	0.516 ± 0.016	0.711 ± 0.071	0.603 ± 0.026	0.573 ± 0.041	0.550 ± 0.018	0.603 ± 0.033
Liver		2.709 ± 0.042	2.550 ± 0.030	2.622 ± 0.062	2.865 ± 0.092	2.677 ± 0.042	3.087 ± 0.119	2.939 ± 0.132	2.864 ± 0.137	3.101 ± 0.106	2.625 ± 0.044	2.898 ± 0.075	2.744 ± 0.080	2.743 ± 0.078	2.983 ± 0.073	2.651 ± 0.030
Spleen		0.274 ± 0.006	0.237 ± 0.009	0.310 ± 0.011	0.316 ± 0.017	0.258 ± 0.012	0.250 ± 0.006	0.226 ± 0.009	0.247 ± 0.009	0.234 ± 0.013	0.220 ± 0.006	0.262 ± 0.005	0.231 ± 0.006	0.278 ± 0.010	0.275 ± 0.014	0.239 ± 0.008
Adrenals		0.025 ± 0.002	0.022 ± 0.001	0.022 ± 0.001	0.023 ± 0.001	0.022 ± 0.001	0.017 ± 0.001	0.012 ± 0.0005	0.016 ± 0.001	0.016 ± 0.001	0.013 ± 0.001	0.021 ± 0.001	0.017 ± 0.0013	0.019 ± 0.001	0.020 ± 0.001	0.017 ± 0.001
Kidneys	L	0.363 ± 0.009	0.324 ± 0.005	0.312 ± 0.007	0.349 ± 0.008	0.321 ± 0.005	0.353 ± 0.006	0.350 ± 0.024	0.335 ± 0.008	0.348 ± 0.008	0.326 ± 0.006	0.358 ± 0.005	0.337 ± 0.012	0.323 ± 0.006	0.349 ± 0.006	0.324 ± 0.004
	R	0.356 ± 0.008	0.323 ± 0.004	0.316 ± 0.008	0.348 ± 0.007	0.330 ± 0.007	0.344 ± 0.008	0.343 ± 0.023	0.335 ± 0.006	0.356 ± 0.006	0.328 ± 0.004	0.350 ± 0.006	0.333 ± 0.012	0.336 ± 0.005	0.352 ± 0.005	0.329 ± 0.004
Testes	L	–	–	–	–	–	0.463 ± 0.007	0.500 ± 0.011	0.474 ± 0.015	0.487 ± 0.016	0.476 ± 0.009	–	–	–	–	–
	R	–	–	–	–	–	0.469 ± 0.006	0.489 ± 0.011	0.498 ± 0.019	0.479 ± 0.018	0.474 ± 0.009	–	–	–	–	–
Epididymedes	L	–	–	–	–	–	0.161 ± 0.006	0.160 ± 0.004	0.149 ± 0.012	0.160 ± 0.006	0.143 ± 0.004	–	–	–	–	–
	R	–	–	–	–	–	0.168 ± 0.007	0.161 ± 0.005	0.167 ± 0.008	0.161 ± 0.004	0.149 ± 0.003	–	–	–	–	–
Uterus + Ovaries		0.299 ± 0.012	0.332 ± 0.026	0.338 ± 0.024	0.309 ± 0.017	0.280 ± 0.016	–	–	–	–	–	–	–	–	–	–

Data presented as mean ± SEM; $n = 10$/sex; one-way ANOVA. No statistically significant difference compared to control.
ANOVA, analysis of variance; bw, body weight; CMC, carboxymethyl cellulose; JJ, Jawarish Jalinoos; SEM, standard error of the mean; SFJJ, sugar-free tablet version of JJ.

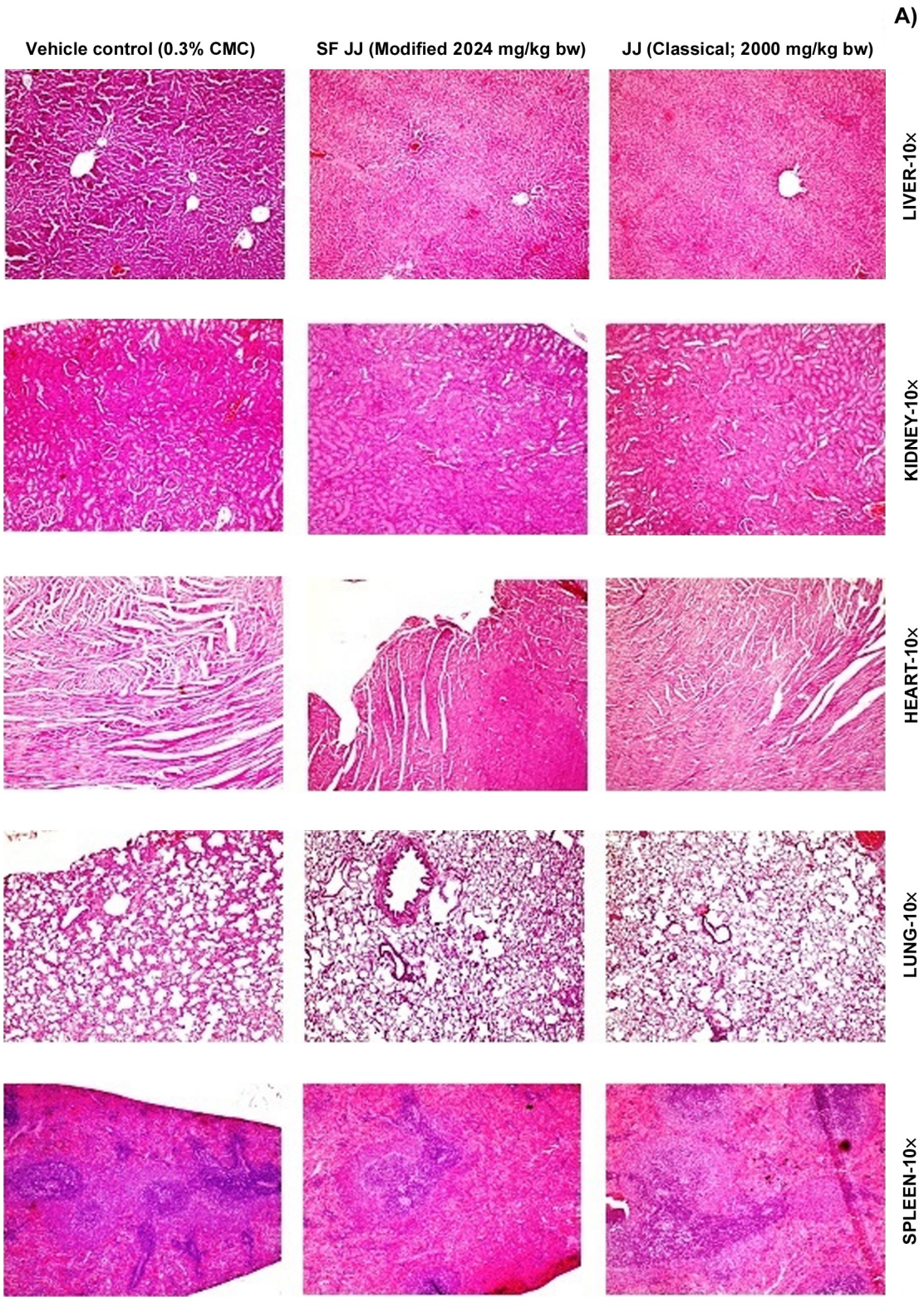

Fig. 5 – Photomicrographs of histological sections. (A) Representative photomicrographs of histological section of vehicle and drug treated rats showing normal architecture of different tissues (H&E stain). (B) Representative photomicrographs of histological section of vehicle and drug treated rats showing normal architecture of different tissues (H&E stain). (C) Representative photomicrographs of histological section of vehicle and drug treated rats showing normal architecture of different tissues (H&E stain).

bw, body weight; CMC, carboxymethyl cellulose; H&E, hematoxylin and eosin; JJ, Jawarish Jalinoos; SFJJ, sugar-free tablet version of JJ.

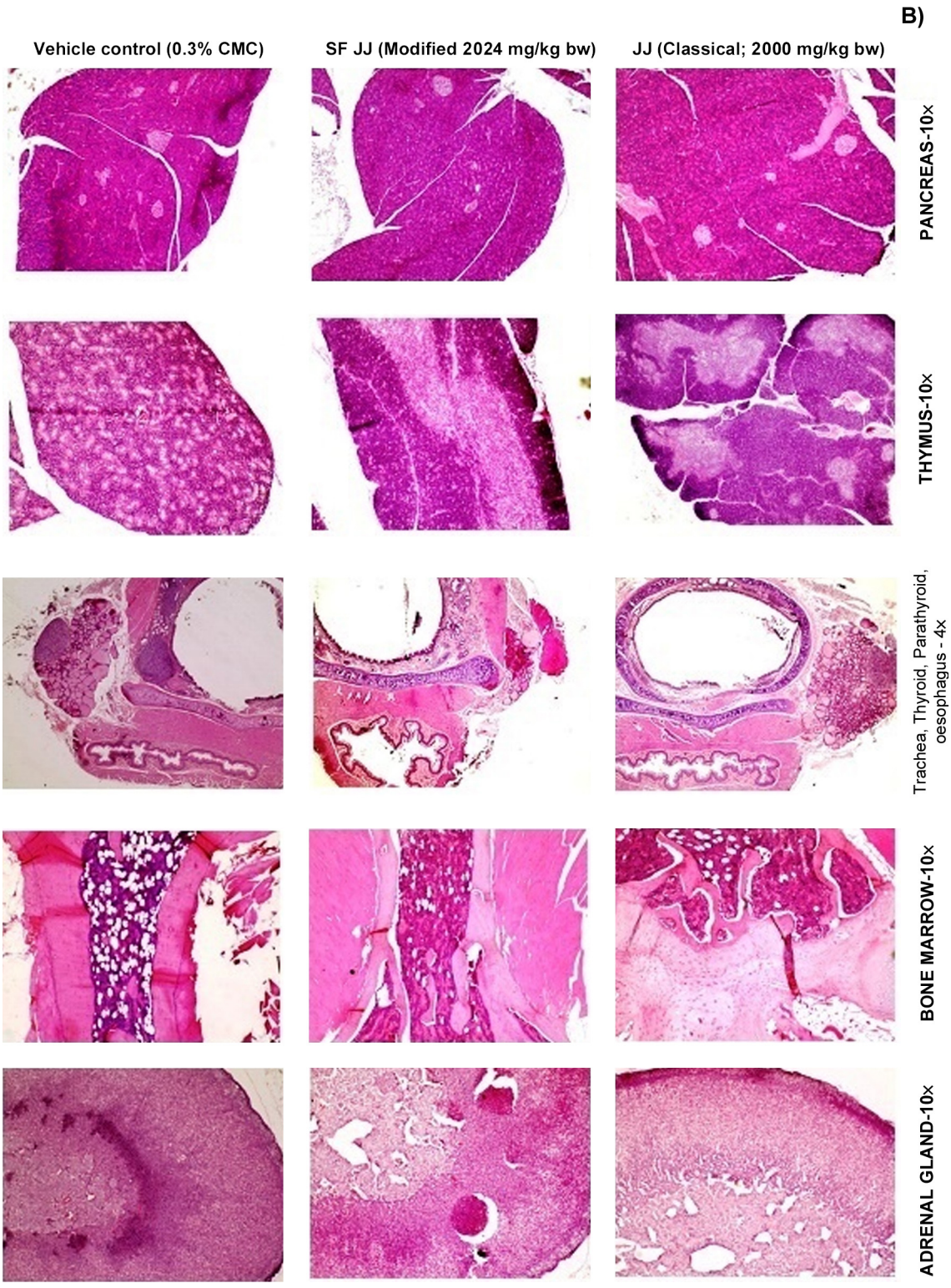

Fig. 5 – (*Continued*)

be influenced by sedatives, muscle relaxants, as well as by toxic substances.[23] The effects of drugs, toxins, muscle relaxants, disease, aging, or neural damage on muscle strength may be assessed using GSM, which automatically measures the grip strength (i.e., peak force) of forelimbs in rats. In the present study, the performance of drug-treated rats was found comparable to that of vehicle-treated rats in rotarod or GSM, indicating the absence of any toxic effect of SFJJ or JJ on motor coordination and muscle grip strength.

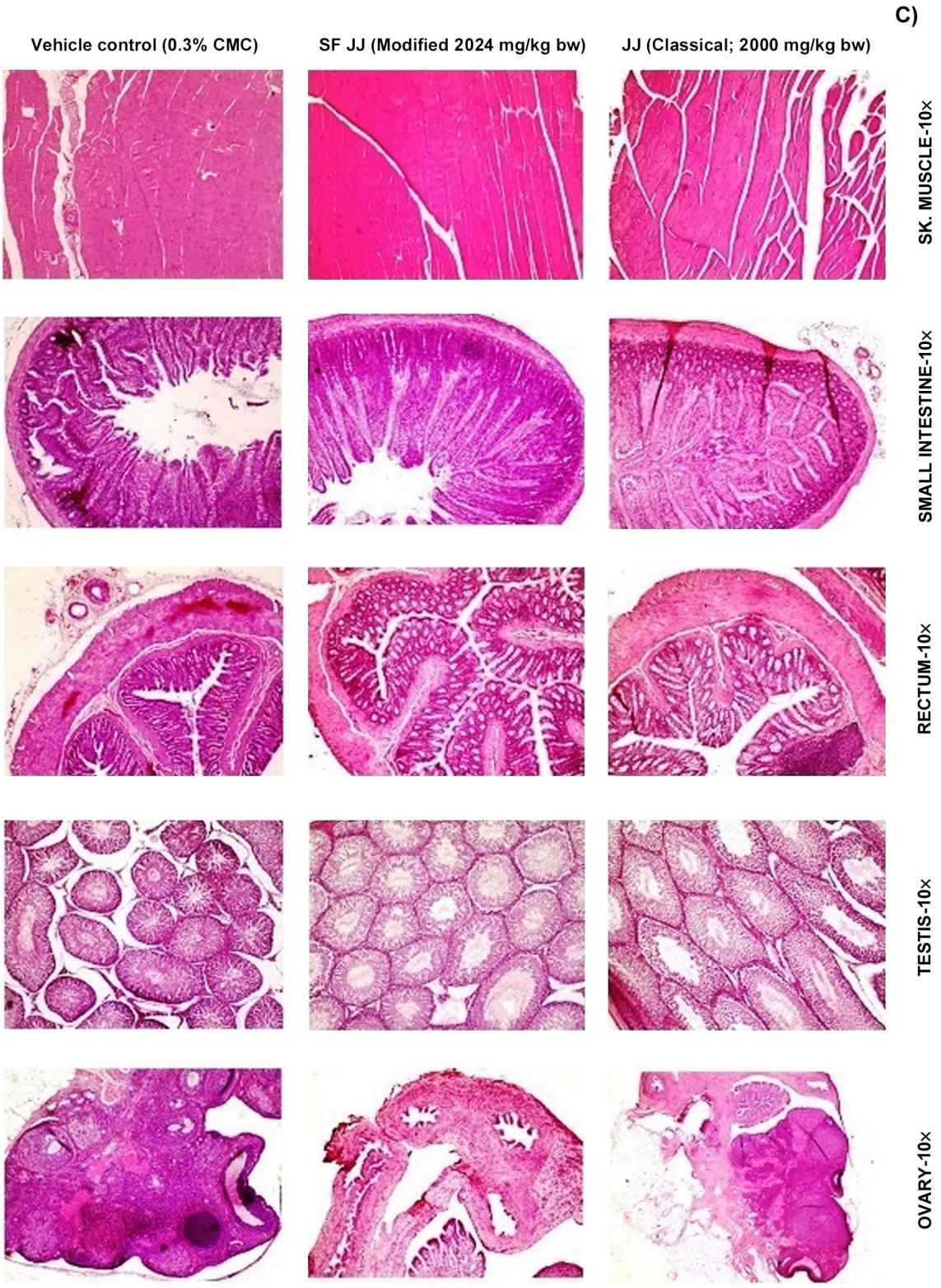

Fig. 5 – (*Continued*)

The evaluation of organ weights in toxicology studies is an integral component in the assessment of new drugs. The Society of Toxicologic Pathology advocates the routine calculation and evaluation of organ/body weight ratios in toxicology studies lasting from 7 days to 1 year.[24] Organ/body weight ratios (i.e., relative organ weight) were considered more useful when body weights were affected.[25] In the present study, relative organ weight data of male and female rats sacrificed at the end of the dosing period was found to be comparable with that of their respective controls. Gross pathological examination did not reveal any abnormal finding. The gold standard of pathology evaluation in toxicity studies has been the examination of paraffin-embedded, hematoxylin and eosin-stained tissue sections.[26] In the present study, no drug treatment-related abnormality was recorded with respect to gross or histological investigation of the organs examined, except for a few changes in the liver of one and two animals in the SFJJ and JJ groups, respectively, which could not be considered toxicologically significant.

As there were no signs of toxicity observed with respect to hematology, clinical chemistry, organ weight, gross, and histological examinations in SFJJ, JJ and control groups, up to the tested dose levels, the No Observed Adverse Effect Level (NOAEL) of SFJJ and classical JJ in SD rats is considered as >2024 mg/kg bw and >2000 mg/kg bw, respectively. Furthermore, based on the present findings, it can be concluded that both classical and sugar-free tablet version of JJ have similar safety profiles.

The present investigation confirms the safety of classical JJ, in line with the long history of its clinical use in the Unani system of medicine, and also establishes the safety of the sugar-free (SFJJ) tablet version.

Conflicts of interests

The authors declare that they have no conflicts of interest.

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